

John Harada, Tamar Lotan, Masaki Ohto,  
Robert B. Goldberg, Robert L. Fischer, Anithu  
Bui, Raymond Kwong  
Application No.: 09/516,052  
Page 2

--The expression cassette comprises a promoter operably linked to the LEC1 polynucleotide or its complement. For example, the promoter can be a constitutive promoter. Alternatively, the promoter can be a promoter from a LEC1 gene. For instance, the LEC1 promoter can be from about nucleotide 1 to about nucleotide 1998 of SEQ ID NO:3. In one embodiment, the heterologous polynucleotide can be linked to the promoter in the antisense orientation. In another embodiment, the promoter is SEQ ID NO:23. The promoter can further comprise SEQ ID NO:24.--

Please replace the paragraph beginning on page 8, line 16 with the following rewritten paragraph.

--A "LEC1 polypeptide" is a sequence of about 50 to about 210, sometimes 100 to 150, amino acid residues encoded by a LEC1 polynucleotide. A full length LEC1 polypeptide and fragments containing a CCAAT binding factor (CBF) domain can act as a subunit of a protein capable of acting as a transcription factor in plant cells. LEC1 polypeptides are often distinguished by the presence of a sequence which is required for binding the nucleotide sequence: CCAAT. In particular, a short region of seven residues (MPIANVI; SEQ ID NO:5) at residues 34-40 of SEQ ID NO:2 shows a high degree of similarity to a region that has been shown to be required for binding the CCAAT box. Similarly, residues 61-72 of SEQ ID NO: 2 (IQECVSEYISFV; SEQ ID NO:6) is nearly identical to a region that contains a subunit interaction domain (Xing, et al., (1993) *EMBO J.* 12:4647-4655).--

Please replace the paragraph beginning on page 12, line 1, with the following rewritten paragraph.

--Figure 1A shows a schematic representation of the three domains of the LEC1 polypeptide. Figure 1B shows a comparison of the predicted amino acid sequence of the B domain encoded by LEC1 (SEQ ID NO:29) with HAP3 homologs from maize (SEQ ID NO:30), chicken (SEQ ID NO:31), lamprey (SEQ ID NO:32), *Xenopus laevis* (SEQ ID NO:33), human (SEQ ID NO:34), mouse (SEQ ID NO:35), rat (SEQ ID NO:35), *Emericella nidulans* (SEQ ID NO: 36), *Schizosaccharomyces pombe* (SEQ ID NO:37), *Saccharomyces cerevisiae* (SEQ ID NO:38), and *Kluyveromyces lactis* (SEQ ID NO:39). The DNA-binding region and the subunit interaction region are indicated. Numbers indicate amino acid positions of the B domains.--

Please replace the paragraph beginning on page 13, line 21, with the following rewritten paragraph.

--Appropriate primers and probes for identifying embryo-specific genes from plant tissues are generated from comparisons of the sequences provided herein. For a general overview of PCR see PCR Protocols: A Guide to Methods and Applications. (Innis, M, Gelfand, D., Sninsky, J. and White, T., eds.), Academic Press, San Diego (1990). Appropriate primers for this purpose include, for instance: UP primer - 5' GGA ATT CAG CAA CAA CCC AAC CCC A 3' (SEQ ID NO: 7) and LP primer 5' GCT CTA GAC ATA CAA CAC TTT TCC TTA 3' (SEQ ID NO:8). Alternatively, the following primer pairs can be used: 5' ATG ACC AGC TCA GTC ATA GTA GC 3' (SEQ ID NO:9) and 5' GCC ACA CAT GGT GGT TGC TGC TG 3' (SEQ ID NO:10) or 5' GAG ATA GAG ACC GAT CGT GGT TC 3' (SEQ ID NO:11) and 5' TCA CTT ATA CTG ACC ATA ATG GTC 3' (SEQ ID NO:12). A third set of primers include: 5'-AGG ATC CAT GGA ACG TGG AGG CTT CCA T-3' (SEQ ID NO:25) and 5'-ATC TAG ATC AGT ACT TAT GTT GTT GAG TCG-3' (SEQ ID NO:26). The amplifications conditions are typically as follows. Reaction components: 10 mM Tris-HCl, pH 8.3, 50 mM potassium chloride, 1.5 mM magnesium chloride, 0.001% gelatin, 200 microM (uM) dATP, 200 microM dCTP, 200 microM dGTP, 200 microM dTTP, 0.4 microM primers, and 100 units per ml Taq polymerase. Program: 96 C for 3 min., 30 cycles of 96 C for 45 sec., 50 C for 60 sec., 72 for 60 sec, followed by 72 C for 5 min.--

Please replace the paragraph beginning on page 17, line 29, with the following rewritten paragraph.

--One of skill will recognize that the polypeptides encoded by the genes of the invention, like other proteins, have different domains which perform different functions. Thus, the gene sequences need not be full length, so long as the desired functional domain of the protein is expressed. As explained above, LEC1 polypeptides are related to CCAAT box-binding factor (CBF) proteins. CBFs are highly conserved family of transcription factors that regulate gene activity in eukaryotic organisms (see, e.g., Mantvani (1992) *Nucl. Acids Res.* 20:1087-1091; Li (1992) *Nucleic Acids Res.* 20:1087-1091). LEC1 was found to have high similarity to a portion of the HAP3 subunit of CBF. Thus, without being bound to any particular theory or mechanism, LEC1 is

likely to act as a transcriptional modulator. HAP3 is divided into three domains, an amino terminal A domain, a central B domain, and a carboxyl terminal C domain, as shown diagrammatically in Figure 1A. Specifically, LEC1, has between about 75% and 85% sequence similarity, which is equivalent to 55% to 63% sequence identity, with the B domains of the other HAP3 homologs shown in Figure 2B; see also, Example 1, below. Figure 1B shows the amino acid sequence homology between LEC1 and other CBF homologs.--

Please replace the paragraph beginning on page 18, line 17, with the following rewritten paragraph.

--The nucleotide sequence for LEC1 corresponding to each domain is displayed in SEQ ID NO:1, *e.g.*, the A domain is located between about nucleotide position 1 to about nucleotide position 81; the B domain is located between about nucleotide position 82 to about nucleotide position 351; the C domain is located between about nucleotide position 352 to about nucleotide position 624.--

Please replace the paragraph beginning on page 18, line 25, with the following rewritten paragraph.

--The DNA binding activity, and, therefore, transcription activation function, of LEC1 polypeptides is thought to be modulated by a short region of seven residues, MPIANVI (SEQ ID NO:5) (found, *e.g.*, at residues 34-40 of SEQ ID NO:2). Thus, the polypeptides of the invention will often retain these sequences.--

Please delete the paragraph beginning on page 23, line 3.

Please replace the paragraph beginning on page 31, line 22, with the following rewritten paragraph.

--The genomic library of *lec1-2* was screened using right and left T-DNA specific probes according to standard techniques. About 12 clones that cosegregate with the mutation, were isolated and purified and the entire DNAs were further labeled and used as probes to screen a southern blot containing wild type and *lec1-1* genomic DNA. One clone hybridized with plant

DNA and was further analyzed. A 7.1 kb XhoI fragment containing the left border and the plant sequence flanking the T-DNA was subcloned into pBluescript-KS plasmid (Stratagene) to form ML7 and sequenced using a left border specific primer (5' GCATAGATGCACTCGAAATCAGCC 3'; SEQ ID NO:13). The T-DNA organization was partially verified using southern analysis with T-DNA left and right borders and PBR322 probes. The results suggested that the other end of the T-DNA is also composed of left border. This was confirmed by generating a PCR fragment using a genomic plant DNA primer (LP primer 5' GCT CTA GAC ATA CAA CAC TTT TCC TTA 3'; SEQ ID NO:8) and a T-DNA left border specific primer (5' GCTTGGTAATAATTGTCATTAG 3'; SEQ ID NO:14) and sequencing.--

Please replace the paragraph beginning on page 32, line 1, with the following rewritten paragraph.

--The EcoRI insert of ML7 was used to screen a wild type genomic library. Two overlapping clones were purified and a 7.4 kb EcoRI genomic fragment from the wild type DNA region was subcloned into pBluescript-KS plasmid making WT74. This fragment was sequenced (SEQ ID NO:4) and was used to screen lec1-1 genomic library and wild type silique-specific cDNA libraries. 8 clones from the lec1-1 genomic library were identified and analyzed by restriction mapping.--

Please replace the paragraph beginning on page 32, line 7, with the following rewritten paragraph.

--From these clones the exact site of the deletion in lec1-1 was mapped and sequenced by amplifying a Xbp PCR fragment using primers (H21 5' CTA AAA ACA TCT ACG GTT CA 3'; SEQ ID NO:15; H 17 - 5' TTT GTG GTT GAC CGT TTG GC 3'; SEQ ID NO:16) flanking the deletion region in lec1-1 genomic DNA. Clones were isolated from both cDNA libraries and partially sequenced. The sequence of the cDNA clones and the wild type genomic clone matched exactly, confirming that both derived from the same locus. All hybridizations were performed under stringent conditions with 32P random prime probes (Stratagene).--

Please replace the paragraph beginning on page 37, line 8, with the following rewritten paragraph.

--Comparison of the deduced amino acid sequence of LEC1 to the GenBank reveals significant similarity to a subunit of a transcription factor, the CCAAT box binding factor (CBF). CBFs are highly conserved family of transcription factors that regulate gene activity in eukaryotic organisms Mantvani, et al., . (1992). Nucl. Acids Res. 20: 1087-1091. They are hetero-oligomeric proteins that consist of between three to four non-homologous subunits. LEC1 was found to have high similarity to CBF-A subunit. This subunit has three domains; A and C which show no conservation between kingdoms and a central domain, B, which is highly conserved evolutionary. Similarly the LEC1 gene is composed of three domains. The LEC1 B domain shares between 75%-85% similarity and 55%-63% identity with different B domains that are found in organisms ranging from yeast to human. Within this central domain, two highly conserved amino acid segments are present. Deletion and mutagenesis analysis in the CBF-A yeast homolog hap3 protein demonstrated that a short region of seven residues (42-48) (LPIANVA; SEQ ID NO:17) is required for binding the CCAAT box, while the subunit interaction domain lies in the region between residues 69-80 (MQECVSEFISFV; SEQ ID NO:18) (Xing et al., supra). LEC1 protein shares high homology to those regions.--

Please replace the paragraph beginning on page 41, line 3, with the following rewritten paragraph.

--The Polymerase Chain Reaction (PCR) was used to amplify the *LIL* gene, which lacks introns. Primers designed to amplify the *LIL* open reading frame contained BamHI and XbaI restriction enzyme sites for cloning purposes. The forward primer, BAMMNJ7-5 sequence is 5'-AGGATCCATGGAACGTGGAGGCTTCCAT-3' (SEQ ID NO:27) with the BamHI site underlined. The reverse primer, 3-MNJ7XBA sequence is 5'-ATCTAGATCAGTACTTATGTTGTTGAGTCG-3' (SEQ ID NO:28) with the XbaI site underlined. The PCR conditions were as follows: 30 cycles of 45 seconds at 94°C, 45 seconds at 53°C, and 3 minutes at 72°C. AmphiTaq DNA polymerase (Perkin Elmer Cetus, 761 Main Ave., Norwalk, CT 06859) was used. PCR products were cloned using the TOPO TA Cloning Kit